0271-0749/02/2205-0496/0
Journal of Clinical Psychopharmacology
Copyright © 2002 by Lippincott Williams & Wilkins, Inc.
Vol. 22, No. 5
Printed in U.S.A.

BRIEF REPORTS

Total Cotinine in Plasma: A Stable Biomarker for Exposure to Tobacco Smoke

JOSE DE LEON, MD*†‡, FRANCISCO J. DIAZ, PHD*, THEA ROGERS, PHARMD*†, DEBRA BROWNE, BS*, LORI DINSMORE, BS*, OMAR H. GHOSHEH, PHD†, LINDA P. DWOSKIN, PHD†, AND PETER A. CROOKS, PHD†

**University of Kentucky Mental Health Research Center at Eastern State Hospital, †University of Kentucky College of Pharmacy, and ‡University of Kentucky College of Medicine, Lexington, Kentucky*

Determination of plasma cotinine concentration is the predominant assay employed to quantify smoking and exposure to environmental tobacco smoke in epidemiological studies. However, cotinine is biotransformed into secondary metabolites. This pilot study determined plasma concentrations of cotinine, cotinine glucuronide, 3-hydroxycotinine, and 3-hydroxycotinine glucuronide. Total cotinine concentration was determined by summation of all four metabolites. The goals of this study were (1) to explore the stability and validity of total cotinine concentration as a measure of tobacco smoking and as a measure of exposure to environmental tobacco smoke in nonsmokers, (2) to explore the stability of plasma concentrations of each of the four nicotine metabolites in smokers by performing a.m. and p.m. measures, and (3) to explore the stability of indices of glucuronidation as measures of possible markers for enzymatic activity. The subject sample included 76 white volunteers (32% smokers and 68% nonsmokers). Plasma total cotinine concentration appeared to be very stable, suggesting that total cotinine concentration may be a good measure for epidemiological studies employing a single plasma sample. Moreover, plasma total cotinine concentration also reflected exposure to environmental tobacco smoke more accurately than did plasma cotinine concentration, which would have not identified 27% of passive smokers.

3-Hydroxycotinine glucuronide and 3-hydroxycotinine plasma concentrations were almost as stable as cotinine concentrations. However, cotinine glucuronide and its indices of glucuronidation were unstable, suggesting that cotinine glucuronide undergoes *in vivo* deconjugation. New studies of total cotinine in plasma using more than two blood collections during the day are needed to definitively establish that it is a stable biomarker for epidemiological studies. (J Clin Psychopharmacol 2002;22:496–501)

Cotinine (COT) is the major metabolite of nicotine, with a relatively long plasma half-life of about 17 hours.[1] Plasma COT concentration is the most widely used measure for quantifying tobacco smoke exposure in clinical and epidemiological studies. Smokers generally have plasma cotinine levels greater than 15 ng/mL (85 nmol/L), while heavy smokers (> 25 cigarettes/day) usually have levels higher than 300 ng/mL (1704 nmol/L).[2] COT is further metabolized to cotinine glucuronide (COTG) and to trans-3′-hydroxycotinine (HCOT), which can also undergo glucuronidation. The sum of these four metabolites—COT, COTG, HCOT, and 3-hydroxycotinine glucuronide (HCOTG)—appears to account for nearly 80% of nicotine metabolism in the average subject. Recently, a simple and efficient analytic method was developed for the quantification of COT, COTG, HCOT and HCOTG,[3] allowing determination of plasma total cotinine (total COT) as a measure of tobacco smoke exposure. Previously, there was a successful attempt to measure urine concentration of these four metabolites.[1] However, the widespread measure of urinary metabolite concentrations in large studies is seriously limited by the necessity to collect urine samples from test subjects over the course of several hours.

Received July 19, 2001; accepted after revision January 29, 2002.
Address requests for reprints to: Jose de Leon, MD, University of Kentucky Mental Health Research Center at Eastern State Hospital, 627 West Fourth St., Lexington, KY 40508. Address e-mail to: jdeleon@uky.edu.

DOI: 10.1097/01.jcp.0000033405.43191.33

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

PM3006723251

Source: https://www.industrydocuments.ucsf.edu/docs/glbj0001

Epidemiological studies mainly employ one biological measurement, usually a single blood sample. In this respect, there are limited data on plasma levels of HCOT[4] and no data on COTG and HCOTG. HCOTG may be a more reliable and more stable measure than COT for assessing tobacco smoke exposure. COT is conjugated by N-glucuronidation, and HCOT is conjugated by O-glucuronidation. Interestingly, black people have slower cotinine metabolism than white people, possible due to differences in glucuronidation of HCOT.[4]

Plasma cotinine concentration is the most widely used and validated biomarker for environmental tobacco smoke (ETS)[5] or secondhand smoke and reflects exposure to nicotine largely from the previous 1 to 2 days. Plasma cotinine levels lower than 15 ng/mL (85 nmol/L) are considered suggestive of exposure to ETS in the absence of smoking.[6] However, there are individual differences in rates and patterns of nicotine and COT metabolism, and depending on the time of plasma collection following exposure to ETS, cotinine may have been metabolized to other secondary metabolites and thus may be undetectable in plasma.

The present pilot study is the first to determine plasma concentrations of four nicotine metabolites—COT, COTG, HCOT, and HCOTG—in smokers and nonsmokers. The plasma concentration obtained by summing [illegible] Several indices of glucuronidation described by Benowitz and associates[1] are investigated as possible measures of enzymatic activity. The goals of this study are (1) to explore the validity of plasma total COT concentration as a measure of tobacco smoke exposure in smokers and as a measure of ETS exposure in nonsmokers, (2) to explore the stability of each of the four metabolites in smokers by performing an a.m. and a p.m. plasma measure, and (3) to explore the stability of indices of glucuronidation as possible biomarkers of enzymatic activity.

Methods

Sample

Subjects signed a consent form approved by two Institutional Review Boards and submitted to two blood collections: a.m. (7:30–9:30 a.m.) and p.m. (2:00–4:00 p.m.). The 76 unpaid white subjects included staff working at a psychiatric hospital, their family members, and volunteers from the community. Seventy percent (53/76) of the subjects were females. Smokers accounted for 32% (24/76) and nonsmokers for 68% (52/76). Nonsmokers included 69% (36/52) who had never smoked and 31% (16/52) who had quit smoking. Sixty-seven percent of nonsmokers (35/52) were female. The respective mean ± SD (range) for age in nonsmokers and smokers was 42 ± 11 (23–63) years and 44 ± 10 (22–62) years. The Fagerstrom Test for Nicotine Dependence was used to measure nicotine dependence.

The accuracy of the self-report on nonsmoking was supported by the subject's background, our personal knowledge of subjects, and the lack of compensation, suggesting altruistic motives. Furthermore, an independent measure of recent smoking status, i.e., carbon monoxide (CO) in expired air, was obtained at the time of collection of the a.m. and p.m. samples. The subjects were informed of the meaning of the CO measurement. None of the nonsmokers had CO values suggestive of recent smoking (all CO results were lower than 10 parts per million, the standard cut-off point for nonsmokers).[6] Respective mean ± SD a.m. and p.m. CO values for smokers were 26.8 ± 17.0 and 23.0 ± 15.8 parts per million. Two additional atypical nonsmokers are described as anecdotal cases to further establish the validity of this new method.

Plasma nicotine metabolite concentrations

Plasma concentrations of COT, COTG, HCOT, and HCOTG were determined using a recently described high-performance liquid chromatographic method.[3] Glucuronide conjugates were determined indirectly: initial basic hydrolysis of the analyte sample was followed by quantification of increase in the resulting deconjugation products. The quantification limits were as follows: COT, 10 ng/mL (57 nmol/L); COTG, 10 ng/mL (27 nmol/L); HCOT, 10 ng/mL (52 nmol/L); and HCOTG, 20 ng/mL (54 nmol/L). The sum of the molar concentrations of the above four metabolites represents the total COT concentration in plasma. Following Benowitz and associates,[1] two indices of glucuronidation were explored for each of the metabolites COT and HCOT. These indices will be referred to as I_1 to I_4 (I_1 = COTG/(COTG + COT); I_2 = COTG/COT; I_3 = HCOTG/(HCOTG + HCOT); I_4 = HCOTG/HCOT). Therefore, I_1 and I_2 are measures of glucuronidation of COT, and I_3 and I_4 are measures of glucuronidation of HCOT.

Statistics

Statistical analyses were performed to explore the plasma stability of total COT concentration, the concentration of each of the four metabolites, and indices of glucuronidation. Determination of the stability of COT is important because most epidemiological studies utilize only one plasma COT measure and assume relative stability throughout the day. Similarly, it is important to determine the stability of COTG, HCOT, and HCOTG concentrations in plasma so that measurements at one time point during the day can be used as a true estimate of their levels over the day. The stability of a particular plasma concentration was analyzed utilizing two approaches: (1) determining the stability of the mean of the

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

PM3006723252

Source: https://www.industrydocuments.ucsf.edu/docs/glbj0001

concentration in the population, and (2) determining the monotonic stability of the concentration. The first approach examined whether the mean value of the concentration in the morning sample was equal to the mean value in the afternoon sample. The second approach examined whether a low (or high) single concentration in the morning was associated with a low (or high) concentration in the afternoon. These two approaches explore different aspects of the plasma concentration stability and were also used to explore the stability of the glucuronidation indices, which are possible measures of enzymatic activity.

Statistical analysis of stability of means

If X is the plasma concentration of a COT metabolite, the total COT concentration or an index of glucuronidation, an important question is whether X is stable—that is, whether X does not vary greatly during the day. To answer this question, two measurements of X, one in the morning (X_{AM}) and one in the afternoon (X_{PM}), were obtained. One approach to answer this question is to study the instability of the mean of X, symbolized by α and defined as the maximum of the two absolute values

$$\left|\frac{E[X_{PM}] - E[X_{AM}]}{E[X_{PM}]}\right| \text{ and } \left|\frac{E[X_{PM}] - E[X_{AM}]}{E[X_{AM}]}\right|,$$

where $E[X_{AM}]$ and $E[X_{PM}]$ denote the population mean of X_{AM} and X_{PM}, respectively. To determine whether this approach is useful, consider the overall daily value of X, which is defined by

$$\mu = E\left|\frac{X_{AM} + X_{PM}}{2}\right|,$$

and notice that

$$|\mu - E[X_{AM}]| \leq \alpha \times E[X_{AM}] \text{ and}$$

$$|\mu - E[X_{PM}]| \leq \alpha \times E[X_{PM}].$$

Thus, if α is not much greater than zero, a single measurement is representative of the overall daily value of X. The percentage error incurred when estimating μ using the mean of a single measurement is not greater than $\alpha \times 100\%$. If α is much greater than zero for a certain population, a single measurement per subject cannot be utilized to study the overall daily value of X in that population, nor can a comparison be made with another population. In this report, α is estimated by substituting $E[X_{AM}]$ and $E[X_{PM}]$ for a.m. and p.m. averages from a particular sample of subjects. Since $\alpha = 0$ if, and only if, $E[X_{PM} - X_{AM}] = 0$, the null hypothesis of stability of the mean of X is tested by comparing the two averages using a paired-sample t-test. If the two-tailed p-value revealed by the test is close to zero, the mean of X is significantly unstable during the day, and the amount of instability is measured by α. The instability α of the mean of the plasma concentration for the four nicotine metabolites, the total COT concentration, and the four indices was estimated using data from 24 smokers. Since the value of α could depend on the population on which the plasma concentration or index is measured, α was also estimated for subpopulations of smokers determined by gender, age, number of cigarettes smoked per day, and number of smokers in the household (Table 1). Paired-sample t-tests were performed using the SPSS statistical package (Chicago, IL) when the difference $X_{PM} - X_{AM}$ was normally distributed according to Shapiro-Wilk tests and Q-Q plots. When the difference $X_{PM} - X_{AM}$ was not normally distributed, p-values were computed by a bootstrap method[7] programmed with Resampling Stats (Arlington, VA).[8] Table 1 provides the values of α in the total sample of smokers and various subsamples. Significant results (with footnotes) suggest lack of stability of the mean and are interpreted as a significant change in the mean of the corresponding population.

Statistical analysis of monotonic stability

The monotonic stability of X (a COT metabolite, the total COT concentration, or an index of glucuronidation) is the tendency for small a.m. measurements to be associated with small p.m. measurements and for large a.m. measurements to be associated with large p.m. measurements. In this study, monotonic stability was assessed by using the Pearson correlation coefficient r between X_{AM} and X_{PM}. The closer r approaches to 0, the more X is considered monotonically unstable, whereas the closer r approaches to 1, the more X is considered stable. Low or moderate values of monotonic stability r can be accompanied by some amount of instability α in the mean. Moreover, a nonsignificant amount of monotonic stability does not imply a significant amount of instability in the mean. If r is very small for a certain population, a single measurement per subject cannot be used to investigate the possible existence of a subpopulation with a very high (or very low) level of X. p-Values for testing the null hypothesis of monotonic instability ($r \leq 0$) were computed using the resampling method implemented in the Resampling Stats software.[8] In addition to coping with small sample sizes, this method has the advantage of not assuming a linear relationship between X_{AM} and X_{PM} in that r may be interpreted just as a measure of monotonic relationship.

Table 2 provides the monotonic stability r in the total sample of smokers and various subsamples. Values are interpreted as any correlation; the closer r is to 1, the greater the monotonic stability. In Table 2, significant results (with footnotes) demonstrate monotonic stabil-

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

PM3006723253

Source: https://www.industrydocuments.ucsf.edu/docs/glbj0001

TABLE 1. Lack of stability of the mean total concentration of nicotine metabolites in plasma, and glucuronidation indices for subpopulations of smokers across the a.m.–p.m. sampling period*

	$\alpha \times 100\%$†								
	Total COT	COT	HCOT	COTG	HCOTG	Index I_1	Index I_2	Index I_3	Index I_4
All	3	3	18	66§	5	49§	132§	15	40¶
Females	1	3	11	59	7	51§	156‡	8	26
Males	7	18	45	80	0.1	44	64	31	68‡
Age ≤ 45 years	11	13	51§	23	6	21	42	30	60‡
Age > 45 years	7	5	4	227§	28‡	137§	478	13	0.2
≤ 20 cigarettes/day	4	10	148	174¶	8	86‡	296	17	58‡
> 20 cigarettes/day	10	19§	24	25	2	28	52	13	23
No other smokers at home	4	2	7	76	6	49	202	1	0.02
Other smokers at home	12	12	82¶	57	4	49§	64‡	30	77¶

*COT, cotinine; COTG, cotinine glucuronide; HCOT, trans-3′-hydroxycotinine; HCOTG, 3-hydroxycotinine glucuronide; I_1, COTG/(COTG + COT); I_2, COTG/COT; I_3, HCOTG/(HCOTG + HCOT); I_4, HCOTG/HCOT; total COT, sum of all four metabolites (COT + HCOT + COTG + HCOTG).

†$\alpha \times 100\%$ indicates percentage of change from a.m. to p.m. mean concentration values.

p-Values are determined by paired-sample *t*-tests (§$0.05 < p \leq 0.1$ border of significance; ‡$0.01 < p \leq 0.05$; ¶$p \leq 0.01$); significant values indicate lack of stability of the mean across the a.m.–p.m. sampling period.

ity. Significant results in Table 1 indicate lack of mean stability.

Results

Plasma concentrations and correlations of metabolites in smokers

The a.m. and p.m. mean ± SD (range) concentrations for COT were 590 ± 362 (0–1272) nmol/L and 572 ± 347 (0–1173) nmol/L, respectively. The a.m. and p.m. mean concentrations for HCOT were 303 ± 174 (0–590) nmol/L and 257 ± 173 (0–530) nmol/L, respectively. The a.m. and p.m. mean concentrations for COTG were 127 ± 148 (0–474) nmol/L and 210 ± 226 (0–766) nmol/L, respectively. The a.m. and p.m. mean concentrations for HCOTG were 195 ± 144 (0–486) nmol/L and 185 ± 146 (0–463) nmol/L, respectively. The a.m. and p.m. correlations between COT and COTG were 0.11 ($p = 0.6$) and 0.10 ($p = 0.7$), respectively. The a.m. and p.m. correlations between HCOT and HCOTG were 0.12 ($p = 0.6$) and 0.40 ($p = 0.1$), respectively. The a.m. and p.m. correlations between COTG and HCOTG were 0.37 ($p = 0.08$) and 0.57 ($p = 0.01$), respectively.

Exploration of plasma total cotinine concentration in smokers

The mean of the plasma total COT concentration did not exhibit significant instability (Table 1). The α for the total sample was 3%, which was not significantly different from 0. Similarly, none of the αs for any subsample was significantly different from 0. When monotonic stability was studied, the plasma total COT concentration was observed to be quite stable in smokers (Table 2). The correlation coefficient between a.m. and p.m. total COT concentration among smokers was $r = 0.92$ ($p < 0.001$). A linear regression analysis of p.m. on a.m. total COT concentration indicated that the intercept and slope of the regression line were not significantly dif-

TABLE 2. Monotonic stability of total cotinine concentration, nicotine metabolites, and glucuronidation indices for subpopulations of smokers across the a.m.–p.m. sampling period*

	r								
	Total COT	COT	HCOT	COTG	HCOTG	Index I_1	Index I_2	Index I_3	Index I_4
All	0.92‡	0.83‡	0.57‡	0.56†	0.82‡	0.52†	0.27	0.68‡	0.63‡
Females	0.91‡	0.83‡	0.59‡	0.44	0.89‡	0.65†	0.27	0.66‡	0.57†
Males	0.89†	0.82†	0.55	0.77	0.41	0.01	0.20	0.79	0.79
Age ≤ 45 years	0.92‡	0.85‡	0.65†	0.69†	0.75‡	0.50	0.57†	0.48	0.47
Age > 45 years	0.94‡	0.83‡	0.55†	0.72†	0.92‡	0.72	0.59	0.88‡	0.90‡
≤ 20 cigarettes/day	0.95‡	0.82‡	0.58†	0.70‡	0.91‡	0.57	0.36	0.74†	0.76†
> 20 cigarettes/day	0.79‡	0.86‡	0.45	0.56	0.55	0.54	0.60	0.67	0.55†
No other smokers at home	0.93‡	0.84‡	0.59†	0.33	0.80‡	0.46	0.28	0.76‡	0.73‡
Other smokers at home	0.93‡	0.84‡	0.75†	0.73†	0.86‡	0.70†	0.72†	0.53	0.64†

*COT, cotinine; COTG, cotinine glucuronide; HCOT, trans-3′-hydroxycotinine; HCOTG, 3-hydroxycotinine glucuronide; I_1, COTG/(COTG + COT); I_2, COTG/COT; I_3, HCOTG/(HCOTG + HCOT); I_4, HCOTG/HCOT; total COT, sum of all four metabolites (COT + HCOT + COTG + HCOTG).

†$0.01 < p \leq 0.05$; ‡$p \leq 0.01$; significant values indicate monotonic stability.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

PM3006723254

Source: https://www.industrydocuments.ucsf.edu/docs/glbj0001

ferent from 0 and 1, respectively, suggesting that a.m. and p.m. total COT concentrations were interchangeable. The respective mean a.m. and p.m. total COT concentrations in smokers' plasma were 1213 ± 653 nmol/L and 1184 ± 636 nmol/L, respectively. Since the total COT concentration was very stable, the average of the a.m. and p.m. total COT concentration was computed for each subject. The mean of this average (1202 ± 624 nmol/L) estimates the overall daily value, μ, described in the statistical section.

Only two smokers had average total COT concentrations equal to 0. These individuals had not smoked between sample collections and reported smoking their last cigarette either 20 hours or 5 days before the a.m. sample. The a.m. and p.m. CO values for these smokers were also 0, consistent with a lack of recent exposure to tobacco smoke. Furthermore, both subjects had Fagerstrom Test for Nicotine Dependence scores of 0, suggesting a lack of nicotine dependence.

Subjects smoking more than 20 cigarettes per day were compared with those who smoked 20 or fewer per day. As expected, the overall daily value of total COT concentration from the group smoking more than 20 cigarettes per day was greater ($\mu = 1508 \pm 415$ nmol/L) than that of the other group ($\mu = 981 \pm 672$ nmol/L). Despite small sample sizes, the difference was significant ($t = 2.1$, $df = 16.7$, one-tailed p-value = 0.03).

Exploration of plasma total cotinine concentration in nonsmokers

The mean ± SD of the subject averages of a.m. and p.m. total COT concentration among the nonsmokers was 24 ± 50 nmol/L, and the median was 0 nmol/L. Almost one third of nonsmokers (29%, 15/52) exhibited an a.m. total COT concentration higher than 0. Seventy-three percent (11/15) of these subjects were identified as having detectable COT levels. Another 27% (4/15) did not have detectable COT but had detectable levels of COT metabolites such that their total COT levels indicated passive exposure to tobacco smoke. A remarkable finding was that a significantly different percentage of quitters (44%, 7/16) compared to never-smokers (14%, 5/36) had a.m. total COT concentrations higher than 0 (Fisher exact test two-tailed, $p = 0.03$).

Exploration of plasma total cotinine concentration in two atypical subjects

A 56-year old man who had recently quit smoking and was wearing a nicotine patch had a total COT concentration of 2626 nmol/L and had detectable values for all four metabolites. A 31-year man who consumed smokeless tobacco had undetectable levels of all four metabolites and stated that he had not consumed any tobacco for 8 hours prior to testing.

Stability of the four nicotine metabolites in plasma

The mean of the COT concentration had low and nonsignificant instability α values among most subpopulations. The subpopulation that smoked more than 20 cigarettes per day had an α value on the border of significance, suggesting the possibility of a lack of stability for that population; however, α was relatively low (19%) (Table 1). Similarly, the r values measuring monotonic stability of COT in this subpopulation were close to 1. When compared with the total COT concentrations, COT concentrations had similar α values (Table 1) and consistently lower r values (eight out of nine) (Table 2). In summary, both measures of stability, α and r values, suggested that plasma total COT concentration is as stable as or even more stable than plasma COT concentration. The mean HCOTG concentration also revealed a nonsignificant or negligible instability α among all subpopulations except subjects older than 45 years of age (Table 1). Moreover, HCOTG exhibited a relatively high monotonic stability except in males and individuals who smoked more than 20 cigarettes per day (Table 2). HCOT concentration was remarkably unstable in smokers who lived with other smokers at home (Table 1). When compared to the plasma concentrations of the other three metabolites, the mean plasma concentration of COTG exhibited significant instability; α was 66% among all smokers (Table 1). Moreover, COTG exhibited a very high instability among subjects older than 45 years and among those smoking fewer than 20 cigarettes per day (227% and 174%, respectively). COTG exhibited the smallest monotonic stability among all smokers ($r = 0.56$) (Table 2).

Stability of glucuronidation indices

The indices of COT glucuronidation, I_1 and I_2, exhibited a very poor monotonic stability (Table 2) and significant αs (Table 1), suggesting lack of stability. The glucuronidation of HCOT was explored using two indices. Index I_3 had α values that did not reach significance, suggesting stability among all subpopulations studied. However, index I_4 was significantly unstable for some subpopulations studied (Table 1). Thus, I_3 appears preferable to I_4 when measuring HCOT glucuronidation, although I_3 must be used cautiously as it does not exhibit significant monotonic stability in some subpopulations (Table 2).

Discussion

Although the current study is limited by small sample size and a lack of subjects from other races, it is the first to attempt to measure COTG, HCOTG, and total COT in the plasma of smokers and nonsmokers. The small sample size precludes the possibility of gender analyses;

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

PM3006723255

Source: https://www.industrydocuments.ucsf.edu/docs/glbj0001

however, Benowitz and associates[1] did not find gender differences in glucuronidation of COT and HCOT.

Cotinine levels had low α values (< 20%). Using a different method (dual stable isotope method), COT levels had average fluctuations of 30% from peak to trough throughout the day.[9]

Our key finding is that plasma total COT concentration exhibited the lowest instability of the mean and the highest monotonic stability when compared to its constituent metabolites. Thus, plasma total COT concentration may be a good biomarker in epidemiological studies using a single measurement. The consistently higher r value of monotonic stability (Table 2) suggested that plasma total COT might be preferable to plasma COT concentration, which has been typically used. Importantly, the plasma total COT concentration may reflect passive exposure to tobacco smoke better than plasma COT concentration alone. If this study had measured only plasma COT concentration, 27% of the passive smokers would have evaded detection. More expensive and sensitive methods (gas chromatography-mass spectrometry and liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry) would have identified more subjects with detectable COT concentrations.[5]

Trans-3′-hydroxycotinine levels in plasma appear to be rather stable following tobacco smoking, although they may be influenced by intensive passive smoke exposure in the home environment. HCOTG plasma concentration was almost as stable as plasma COT concentration. It was remarkable that COTG had much lower stability compared to the other three metabolites.

One of the indices of glucuronidation of HCOT, e.g., I_3, had good stability, suggesting that it may be worthwhile to explore its utility as a measure of polymorphic variations in the glucuronidation of HCOT. The low stability of plasma COTG concentration and its indices of glucuronidation are worthy of comment. Some glucuronides exhibit *in vivo* lability. Thus, it is possible that COTG may undergo *in vivo* deconjugation by β-glucuronidase. Consequently, if COTG is susceptible to deconjugation, this susceptibility may explain its low plasma stability. It is not known whether one or two uridine-5′-diphospho-α-D-glucuronosyltransferase enzymes are responsible for the N-1-glucuronidation of COT and the 3′-O-glucuronidation of HCOT. In this respect, the results from the present study as well as from previously published data[1] suggest that different glucuronosyltransferase enzymes are involved.

Conclusions

The current results suggest that plasma total COT may be a useful, stable biomarker for determining exposure to tobacco smoke, including ETS. New studies of total COT in plasma using more than two blood collections during the day are needed to establish definitively that this measure is a stable biomarker for epidemiological studies.

Acknowledgements

This study was supported in part by grants from the University of Kentucky Medical Center Research Fund (J.d.L.); MH-51380 from the National Institute of Mental Health (J.d.L.); DA-08656 (L.D. and P.A.C.) and DA-00399 (L.D.) from the National Institute of Drug Abuse; a National Alliance for Research in Schizophrenia and Depression (NARSAD) Young Investigator Award (T.R.); and a supplement to the NARSAD grant from the Department of Mental Health and Mental Retardation/Department of Psychiatry, University of Kentucky, through the University Collaboration Project (T.R.). F.J.D. is Associate Instructor in Statistics at Universidad Nacional de Colombia. F.J.D. was supported by a grant from the Kentucky Department of Mental Health and Mental Retardation and the Department of Psychiatry of the University of Kentucky through the University Collaboration Project (J.d.L.). O.H.G. was a student in the Pharmaceutical Sciences PhD program at the University of Kentucky when he took part in this study. Jenny Boyle, BS; Jimmie Brumagen, RN; Lynn Weddle, RN; and Meg Susce, RN, helped with data collection. Meg Susce, RN, helped with editing.

References

1. Benowitz NL, Perez-Stable E, Fong I, et al. Ethnic differences in N-glucuronidation of nicotine and cotinine. J Pharmacol Exp Ther 1999;291:1196–203.
2. Woodward M, Tunstall-Pedoe H, Smith WCS, et al. Smoking characteristics and inhalation biochemistry in the Scottish population. J Clin Epidemiol 1991;44:1405–10.
3. Ghosheh O, Browne D, Rogers T, et al. A simple high performance liquid chromatographic method for the quantification of total cotinine, total 3′-hydroxycotinine and caffeine in the plasma of smokers. J Pharm Biomed Anal 2000;23:543–9.
4. Benowitz NL, Jacob P. Trans-3′-hydroxicotinine: disposition kinetics, effects and plasma levels during cigarette smoking. Br J Clin Pharmacol 2001;51:53–9.
5. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol Rev 1996;18:188–204.
6. Cummings SR, Richard RJ. Optimum cutoff points for biochemical validation of smoking status. Am J Public Health 1991;78:574–5.
7. Efron B, Tibshirani RJ. An introduction to the bootstrap. New York: International Thomson Publishing, 1993.
8. Simon JL. Resampling Stats user's guide. Arlington, VA: Resampling Stats, 1999.
9. Benowitz NL, Jacob P. Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Pharmacol Ther 1994;56:483–93.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

PM3006723256

Source: https://www.industrydocuments.ucsf.edu/docs/glbj0001